Kindly amend the application as follows.[1]

IN THE SPECIFICATION

Replace the paragraph on page 1, beginning on line 12, down to line 28 (the last line on the page) with the following paragraph.

PCT application WO 94/02602, published 3 February 1994 and incorporated herein by reference, describes in detail the production of transgenic nonhuman animals which are modified so as to produce antibodies with fully human variable regions rather than endogenous antibodies in response to antigenic challenge. Briefly, the endogenous loci encoding the light and heavy immunoglobulin chains are incapacitated in the transgenic hosts and loci encoding human heavy and light chain proteins are inserted into the genome. In general, the animal which provides all the desired modifications is obtained by cross-breeding intermediate animals containing fewer than the full complement of modifications. The preferred embodiment of the nonhuman animal described in the specification is a mouse. Thus, mice, specifically, are described which, when administered immunogens, produce antibodies with human variable regions, including fully

Replace the paragraph on page 2, lines 14-18 with the following paragraph.

The availability of the nonhuman, immunogen-responsive transgenic animals described in the above-referenced WO

1 An "Appendix of Amendments" is enclosed herewith showing the amendments to the specification. In the Appendix, additions are underscored and deletions are bracketed.

94/02602 makes possible convenient production of human antibodies without the necessity of employing human hosts.

Replace the paragraph on page 8, lines 3-5 with the following paragraph.

The details for constructing such an animal useful in the method of the invention are provided in the PCT application WO 94/02602 referenced above.

Replace the paragraph on page 20 beginning at line 18 down to line 31 (the last line on the page) with the following paragraph.

In these examples, mice, designated "xenomice", are used for initial immunizations. A detailed description of such xenomice is found in the above-referenced PCT application WO 94/02602. Immunization protocols appropriate to each antigen are described in the specific examples below. The sera of the immunized xenomice (or the supernatants from immortalized B cells) were titrated for antigen specific human antibodies in each case using a standard ELISA format. In this format, the antigen used for immunization was immobilized onto wells of microtiter plates. The plates were washed and blocked and the sera (or supernatants) were added as serial dilutions for 1-2 hours of incubation. After washing, bound antibody having human characteristics was

At page 39, before the claims, insert the following paragraphs

CLONING OF HUMAN HEAVY CHAIN LOCUS USING YEAST ARTIFICIAL CHROMOSOMES

A. PRODUCTION OF YEAST ARTIFICIAL CHROMOSOME (YAC) CONTAINING HUMAN HEAVY CHAIN

An SpeI fragment, spanning the human heavy chain VH6-D-J-Cμ-Cδ region (Berman *et al.* (1988) , *EMBO J.* 7: 727-738; see Figure 20) is isolated from a human YAC library (Burke, *et al.*, *Science, 236;* 806-812) using DNA probes described by Berman *et al.* (1988) *EMBO J.* 7:727-738. One clone is obtained which is estimated to be about 100 kb. The isolated YAC clone is characterized by pulsed-field gel electrophoresis (Burke *et al.*, supra; Brownstein *et al.*, *Science, 244*: 1348-1351), using radiolabelled probes for the human heavy chain (Berman *et al.*, *supra*).

B. INTRODUCTION OF YAC CLONES INTO EMBRYOS OR ES CELLS

High molecular weight DNA is prepared in agarose plugs from yeast cells containing the YAC of interest (i.e., a YAC containing the aforementioned SpeI fragment from the IgH locus). The DNA is size-fractionated on a CHEF gel apparatus and the YAC band is cut out of the low melting point agarose gel. The gel fragment is equilibrated with polyamines and then melted and treated with agarase to digest the agarose. The polyamine-coated DNA is then injected into the male pronucleus of fertilized mouse embryos which are then surgically introduced into the uterus of a psueudopregnant female as described above. The transgenic nature of the newborns is analyzed by a slot-blot of DNA isolated from tails and the production of human heavy chain is analyzed by obtaining a small amount of serum and testing it for the presence of Ig chains with rabbit anti-human antibodies.

As an alternative to microinjection, YAC DNA is transferred into murine ES cells by ES cell: yeast protoplast fusion (Traver *et al.* , (1989) *Proc. Natl. Acad. Sci.*, USA, 86: 5898-5902; Pachnis *et al.*, (1990), *ibid 87;* 5109-5113). First, the neomycin-resistance gene from pMClNeo or HPRT or

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other mammalian selectable marker and a yeast selectable marker are inserted into nonessential YAC vector sequences in a plasmid. This construct is used to transform a yeast strain containing the IgH YAC, and pMClNeo (or other selectable marker) is integrated into vector sequences of the IgH YAC by homologous recombination. The modified YAC is then transferred into an ES cell by protoplast fusion (Traver *et al.* (1989); Pachnis *et al.*, 1990), and resulting G418-resistant ES cells (or exhibiting another selectable phenotype) which contain the intact human IgH sequences are used to generate chimeric mice. Alternatively, a purified YAC is transfected, for example by lipofection or calcium phosphate-mediated DNA transfer, into ES cells.

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PRODUCTION OF HUMAN IG BY CHIMERIC MICE BY INTRODUCTION OF HUMAN IG USING HOMOLOGOUS RECOMBINATION

As an alternative approach to that set forth in Examples I-VI, human Ig genes are introduced into the mouse Ig locus by replacing mouse heavy and light chain immunoglobulin loci directly with fragments of the human heavy and light chain loci using homologous recombination. This is followed by the generation of chimeric transgenic animals in which the embryonic stem-cell derived cells contribute to the germ line.

A. CONSTRUCTION OF HUMAN HEAVY CHAIN REPLACEMENT VECTOR.

The replacing human sequences include the SpeI 100 kb fragment of genomic DNA which encompasses the human VH6-D-J-Cμ-Cδ heavy chain region isolated from a human-YAC library as described before. The flanking mouse heavy chain sequences, which drive the homologous recombination replacement event, contain a 10 kb BamHI fragment of the mouse Cε-Cα heavy chain and a 5' J558 fragment comprising the 5' half of the J558 fragment of the

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mouse heavy chain variable region, at the 3' and 5' ends of the human sequences, respectively (Figure 20). These mouse sequences are isolated from a mouse embryo genomic library using the probes described in Tucker *et al.* (1981) , *PNAS USA. 78;* 7684-7688 and Blankenstein and Krawinkel (1987, *supra*) , respectively. The 1150 bp XhoI to BamHI fragment, containing a neomycin-resistance gene driven by the Herpes simplex virus thymidine kinase gene (HSV-tk) promoter and a polyoma enhancer is isolated from pMClNeo (Koller and Smithies, 1989, *supra*) . A synthetic adaptor is added onto this fragment to convert the XhoI end into a BamHI end and the resulting fragment is joined to the BamHI mouse Cε-Cα in a plasmid.

From the YAC clone containing the human heavy chain locus, DNA sequences from each end of the insert are recovered either by inverse PCR (Silverman *et al.* (1989) , *PNAS, 86*:7485-7489) , or by plasmid rescue in *E. coli*. Burke *et al.*, (1987); Garza *et al.* (1989) *Science, 246*:641-646; Traver *et al.*, 1989) (see Figure 20). The isolated human sequence from the 5'V6 end of the YAC is ligated to the mouse J558 sequence in a plasmid and likewise, the human sequence derived from the 3'Cd end of the YAC is ligated to the Neo gene in the plasmid containing Neo and mouse Cε-Cα described above. The human V6-mouse J558 segment is now subcloned into a half-YAC cloning vector that includes a yeast selectable marker (*HIS3*) not present in the original IgH YAC, a centromere (CEN) and a single telomere (TEL). The human Cδ-Neo-mouse Cε-Cα is likewise subcloned into a separate half-YAC vector with a different yeast selectable marker (*LEU2*) and a single TEL. The half-YAC vector containing the human V6 DNA is linearized and used to transform a yeast strain that is deleted for the chromosomal *HIS3* and *LEU2* loci and which carries the IgH YAC. Selection for histidine-prototrophy gives rise to yeast colonies that have undergone homologous recombination between

the human V6 DNA sequences and contain a recombinant YAC. The half-YAC vector containing the human Cδ DNA is then linearized and used to transform the yeast strain generated in the previous step. Selection for leucine-prototrophy results in a yeast strain containing the complete IgH replacement YAC (see Figure 20). Preferably, both targeting events are performed in a single transformation step, selecting simultaneously for leucine and histidine prototrophy. This is particularly useful when the original centric and acentric YAC arms are in opposite orientation to that shown in Figure 20. This YAC is isolated and introduced into ES cells by microinjection as described previously for embryos.

IN THE DRAWINGS

Add Figure 20 attached hereto after Figure 19B.

REMARKS

Applicants have amended the specification at pages 1, 2, 8, and 20 to remove reference to pages 39-141.

Applicants have amended the specification at page 39 to insert paragraphs from WO 94/02602. The first three paragraphs correspond to the disclosure on page 54, line 23 to page 55, line 36 of WO 94/02602. The following three paragraphs correspond to the disclosure on page 71, line 1 to page 72, line 32 of WO 94/02602.

Applicants have amended the drawings to add a figure referenced to in the paragraphs from WO 94/02602 inserted into this application.

The disclosure of WO 94/02602 was incorporated by reference in the application as filed. Applicant's response is accompanied by a Declaration of R. Minako Pazdera, applicant's agent, stating that the amendatory material